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Nephropathy of type II diabetes: Evidence for hereditary factors?

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Nephropathy of type II diabetes: Evidence for hereditary factors? Family studies point to an important genetic element in the genesis of diabetic nephropathy, but it is not known whether renal abnormalities are present prior to the onset of diabetes. To address this issue we examined all consecutive patients suffering from type II diabetes with a duration of more than 10 years who attended a diabetes outpatient clinic. Ninety-four patients had nephropathy, 307 did not. All offspring who were phenotypically normal (no hypertension, normal oral glucose tolerance, non-smoking) and agreed to participate were examined, 26 from nephropathic and 30 from non-nephropathic diabetic parents. They were compared with 30 offspring matched for age, gender and BMI from non-diabetic parents as controls. We measured urinary albumin excretion under baseline conditions and at several time points after ingestion of 300 g cooked beef and submaximal treadmill exercise, respectively. In addition, casual blood pressure, ambulatory blood pressure, urinary albumin and urinary alpha-1-microglobulin were measured. Primary renal disease was excluded by clinical examination. Under baseline conditions, median urinary albumin excretion rate (AER; μg/min) was significantly ($P < 0.005$) higher in offspring of nephropathic type II diabetic patients (7.8; range 1.04 to 19.5) than in the offspring of non-nephropathic type II diabetic patients (4.8; 0.36 to 17.5) and controls (4.4; 0.16 to 18.4). Submaximal treadmill exercise caused a greater proportional increase of AER in offspring of nephropathic type II diabetics (median 16-fold) than in offspring of non-nephropathic diabetic patients (6.3-fold) or controls (4.8-fold). In offspring of nephropathic diabetic patients casual and particularly ambulatory systolic blood pressures were significantly higher, but AER was not correlated with blood pressure. In summary, higher values, albeit within the normal range, for baseline and postexercise albuminuria were noted in phenotypically normal offspring of parents with type II diabetes and nephropathy. The observation suggests that changes in transglomerular albumin traffic are demonstrable prior to the onset of diabetes and diabetic nephropathy in subjects with a potential genetic predisposition to these conditions.

There is convincing evidence for a genetic basis of type II diabetes [1]. Diabetic nephropathy is observed in up to 30% of patients who suffer from type II diabetes for more than 10 years [2, 3]. Familial aggregation of nephropathy in type II diabetes has been noted both in Pima Indians [4] and Caucasoids [5]. This observation is consistent with, but not definite evidence for the hypothesis that independent genes code for type II diabetes on the one hand and development of diabetic nephropathy on the other hand.

Phenotypically normal offspring of parents with type II diabetes have marked abnormalities of insulin and glucose metabolism even when they are still normotensive and exhibit no glucose intolerance [6, 7].

Little is known, however, concerning potential early abnormalities of albumin excretion in offspring of parents with type II diabetes and diabetic nephropathy. In one study [8], higher urinary albumin excretion rates were noted in the offspring of diabetic patients with type II diabetes, but detailed investigations of the response of albuminuria to interventions were not performed in this cohort for which subjects had not been randomly selected.

To further address this issue, we examined a large cohort of adult subjects with type II diabetes of long duration both with and without nephropathy, excluding MODY and patients suggestive of mitochondrial diabetes. Presence of nephropathy was diagnosed on the basis of elevated urinary albumin excretion.

In a second step we examined all available offspring measuring (i) blood pressure and (ii) urinary albumin excretion under baseline conditions and under stimulatory maneuvers.

Methods

Description of study cohorts

All consecutive type II diabetic patients who visited the outpatient clinic in Zabrze (Poland) either *de novo* or for routine controls between November 1994 and June 1995 were identified. Type II diabetes was diagnosed according to the criteria of the National Diabetes Data Group (NDDG) [9]. Only patients with known duration of type II diabetes for more than 10 years were considered for further workup (that is, diabetes of sufficient duration to allow development of nephropathy). Standard chronic primary renal disease was excluded by urine analysis (phase contrast microscopy, bacterial culture), renal ultrasonography and clinical workup. Of the 425 patients, 24 were excluded because of primary chronic renal disease. Diabetic nephropathy, that is, > 30 mg albumin/24 hr on at least two of three occasions with or without elevated serum creatinine was present in 94 and absent in 307 patients. Of the former, 14 had no children and in 33 children were not locally available for study. The respective numbers for the latter group were 26 and 163. Exclusion criteria for the offspring were hypertension, that is, > 140/90 mm Hg under resting conditions, and glucose intolerance, that is, > 140 mg/dl two hours after 75 g oral glucose. Furthermore, smokers and patients on oral contraceptives were excluded. After exclusion of offspring who refused cooperation ($N = 86$) or who met exclusion criteria ($N = 23$) the study concerned the families of 56 parents

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Table 1. Characteristics of study subjects

	Offspring of parents with		
	Diabetes no diabetic nephropathy (N = 30)	Diabetes plus diabetic nephropathy (N = 26)	No diabetes (controls) (N = 30)
Age *years*	33.0 ± 8.5	33.0 ± 6.5	31.5 ± 5.4
Gender *male/female*	15/15	14/12	15/15
BMI *kg/m²*	26.2 ± 5.1	26.6 ± 3.4	25.5 ± 3.0
BMI > 25 (>30 kg/m²)	7 (7)	16 (4)	15 (2)
HbA1c %	4.85 ± 0.46	5.23 ± 0.33	4.90 ± 0.38
MAP *mm Hg*	87 ± 8.6	90 ± 10.2	85 ± 0.00
Total cholesterol *mmol/liter*	4.87 ± 1.14	4.88 ± 1.16	4.41 ± 0.91
Triglycerides *mmol/liter*	1.01 ± 0.65	1.06 ± 0.59	0.91 ± 0.32
Urine urea nitrogen *mmol/day*	340 ± 50	330 ± 47	321 ± 37
Na excretion *mmol/24 hr*	150 ± 24	145 ± 23	148 ± 19.2

(median 59 years, range 51 to 76; 19 fathers, 37 mothers) who had type II diabetes of more than 10 years duration and whose children were locally available for the study. Diabetic nephropathy was present in 26 of the parents (11 fathers, 15 mothers) and absent in 30 (8 fathers, 22 mothers). Established hypertension, that is, blood pressure > 160/95 mm Hg or antihypertensive treatment, was present in 21 of 26 nephropathic and 14 of 30 non-nephropathic parents.

Study subjects (offspring of diabetic and non-diabetic parents)

We examined 56 offspring of the above diabetic patients. The relevant demographic and clinical data are summarized in Table 1.

Among the offspring of diabetic parents without nephropathy, diabetes was present on the father's side in 8 and on the mothers's side in 22. The respective numbers for offspring of diabetic parents with nephropathy were 11 on the father's side and 15 on the mother's side. None of the offspring of diabetic mothers had evidence of mitochondriopathy.

In the subsequent analyses no significant difference was noted according to whether diabetes was present in the father or the mother.

Despite considerable effort no information could be obtained on birth weight of the propositi.

As controls we examined 30 local offspring of non-diabetic parents without history of hypertension. The controls were recruited from the staff of the diabetes clinic and matched for age, gender and body mass index.

Study protocol

All subjects collected overnight urine for analysis of albumin, alpha-1-microglobulin and creatinine. They were seen in the outpatient clinic at 9 a.m. and passed urine. They stayed in a quiet room in supine position except for passing urine. At 9 a.m. they had an oral water load (10 ml/kg); urine was collected and BP was measured at 15 minute intervals. At 10 hours they ingested 300 g cooked beef and urine collections and BP measurements were continued for three hours.

Patients were asked to drink an amount of water that corresponded to the volume of urine passed.

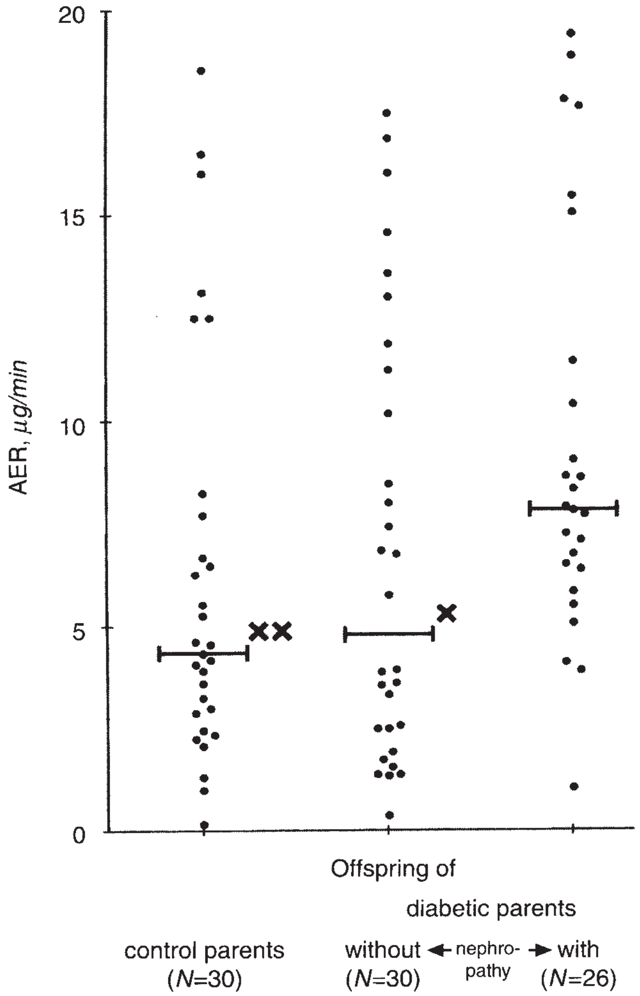


Fig. 1. *Night time albumin excretion rate (AER) in offspring of control parents and diabetic parents without and with diabetic nephropathy (DN).* Significant difference between offspring of parents with DN and without DN (x $P < 0.05$) or DN and controls (xx $P < 0.005$).

On a second day, the individuals were subjected to a submaximal treadmill exercise test at 80% of target heart rate. Urine was collected immediately after exercise, one hour after exercise and subsequently after night time rest.

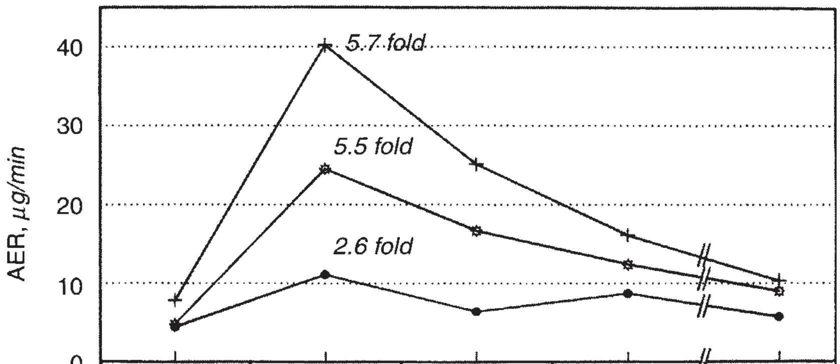
Ambulatory blood pressure was measured on a work day several weeks after the study under outpatient conditions using a Medilog ABP (Oxford, UK). Patients wrote a protocol to document their activities and time of bed rest.

Measurements

Urinary albumin by was measured by radioimmunoassay [10]. Urinary alpha-1-microglobulin was measured by laser nephelometry [11], urinary creatinine by spectrophotometry, hemoglobin A1C by immunoagglutination inhibition using a DCA-2000 autoanalyzer (Bayer Diagnostics, Elkhard, IN, USA), polymorphisms of the genes of the renin angiotensin system (ACE, angiotensinogen, AT_1 receptor) according to [12–14].

Statistics

End points were defined prior to the study as: baseline albumin excretion in morning urine; albumin excretion after ingestion of



Basal	1 hr	2 hr	3 hr	21 hr
4.4 (0.2–18.5)	****11.1 (2.3–108)	****6.4 (0.3–91)	*8.7 (1.0–156)	*5.7 (0.5–58)
4.8 (0.4–17.5)	**24.5 (4.2–227)##	** 16.7 (2.8–589)	12.4 (1.9–109)	9.0 (0.5–58)
7.8 (1.1–19.5)	40.2 (11–431)	25.1 (4.1–105)	16.1 (3.6–288)	10.3 (3.5–59)

Fig. 2. *Time course of albumin excretion rate (AER) after protein meal ingestion in offspring of control parents and diabetic parents without and with diabetic nephropathy (DN).* Note the 5.7-fold increase in offspring of DN ($P < 0.005$ vs. controls) and 5.5-fold in the offspring of no DN ($P < 0.05$ vs. controls). Symbols are: (●) controls; (○) offspring no DN; (+) offspring DN; *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$; ****$P < 0.001$ vs. offspring of diabetic patients. ##$P < 0.01$. vs. control offspring.

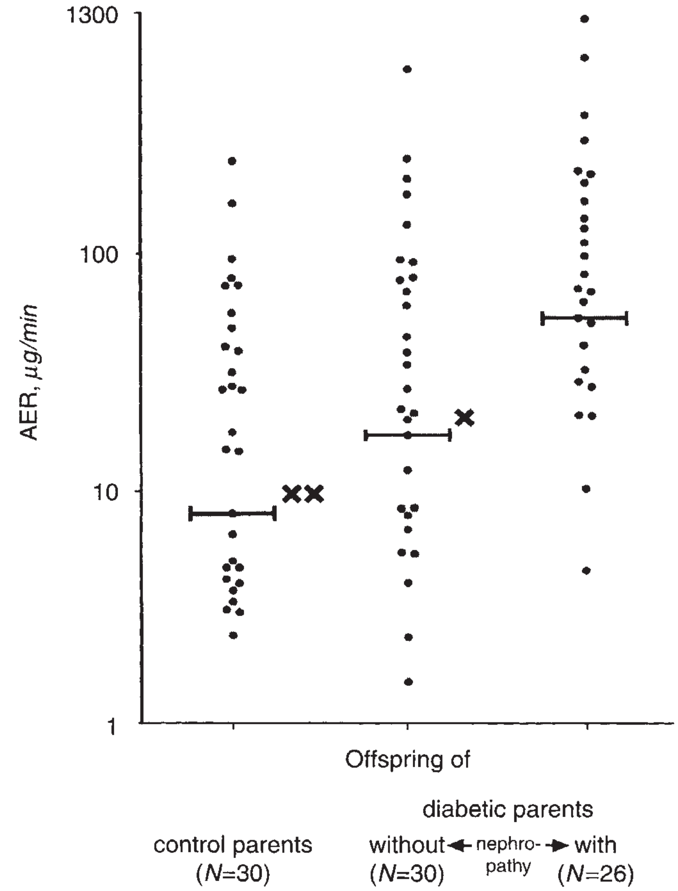


Fig. 3. *Albumin excretion rate (AER) after submaximal treadmill exercise in offspring of control parents and diabetic parents without and with diabetic nephropathy (DN).* Significant difference between offspring of parents with DN and without DN (x $P < 0.01$) or DN and controls (xx $P < 0.001$).

beef; and albumin excretion during physical exercise. Significance was determined at $P < 0.05$ by Wilcoxon's test or ANOVA for repeated measures as indicated (SAS program). Further exploratory analyses were performed using Wilcoxon's test. Such exploratory analyses were accepted as significant after Bonferroni correction. Data are given as x ± SD (if normally distributed) or median and range (if not normally distributed).

Results

Night time urinary albumin excretion in offspring of diabetic parents with or without nephropathy

As shown in Figure 1, significantly higher albumin excretion rates (AER), although not beyond the normal range (< 20 μg/min) [15], were noted in offspring of diabetic parents with nephropathy. Alpha-1-microglobulin levels were below the upper 95th percentile of normal values, that is, < 110 mg/24 hr in all patients. For albumin and alpha-1-microglobulin, the CVs of replicate measurements were < 5% and 7%, respectively.

Following a water load (10 ml/kg) AER increased to a similar proportion in controls (from 4.4 μg/min, range 0.16–18.5 to 18.4; 2.6–108, that is, 4.8-fold), in offspring of nephropathic diabetic parents (from 6.8; 1.04–15.5 to 46.6; 3.2–502, that is, 6.4-fold) and non-nephropathic diabetic parents (4.8; 0.36–17.5 to 24.8; 1.7–88.7, that is, 3-fold). There was no significant difference in the proportional increase between the groups.

Urinary albumin excretion rate after protein meal ingestion

As shown in Figure 2, AER increased significantly in all three groups following ingestion of 300 g cooked beef. The proportional increase at one hour was significantly higher in offspring of diabetic parents irrespective of nephropathy when compared with offspring of non-diabetic parents. The relative changes and result of statistical analysis were similar, when albumin/creatinine ratios were used instead of albumin excretion rates.

AER was still higher three hours after ingestion, but returned to baseline after 21 hours.

Albumin excretion after treadmill exercise

As shown in Figures 3 and 4, AER was significantly higher in offspring of nephropathic diabetic parents compared to offspring of non-nephropathic parents or controls. The proportional increase of albumin/creatinine ratio (mg/mmol) was significantly higher in offspring of nephropathic diabetic parents (median 16-fold; range 1.2 to 236; $P < 0.01$ vs. controls) than in offspring of non-nephropathic diabetic parents (median 6.3-fold; 1.5 to 231) or offspring of non-diabetic parents (median 4.8; 1.2 to 99).

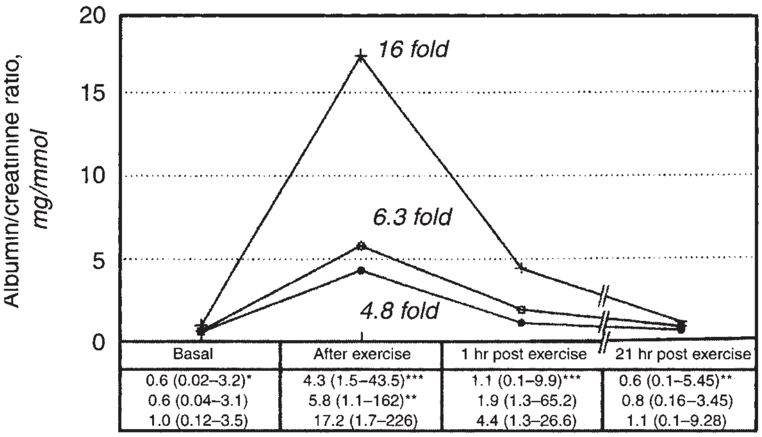


Fig. 4. *Time course of albumin/creatinine ratio after submaximal treadmill exercise.* Note 16-fold increase in offspring of DN ($P < 0.01$ vs. controls) and 6.3-fold increase in offspring of no DN (not significant vs. controls). Symbols are: (●) controls; (○) offspring no DN; (+) offspring DN; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$; $^{****}P < 0.001$ vs. offspring of diabetic patients. $^{\#\#}P < 0.01$. vs. control offspring.

Table 2. Casual and ambulatory blood pressure (mm Hg) in offspring according to presence of diabetes and diabetic nephropathy of parents

	Casual BP		24 hr BP		Daytime BP		Sleeping time BP	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic
Offspring of control parents ($N = 30$)	112 ± 10.3	72 ± 8.5	114 ± 8.5	71.6 ± 6.4	117 ± 9.3	74 ± 6.5	106 ± 9.1	66 ± 7.9
Offspring of diabetic parents without nephropathy ($N = 30$)	114 ± 10.3	73 ± 8.5	117 ± 12.9	74 ± 8.6	120 ± 13.2	77 ± 8.4	107 ± 12.8	69 ± 10.5
with nephropathy ($N = 26$)	121 ± 16.7[ab]	76 ± 8.7	125 ± 16.9[ab]	76 ± 9.2	127 ± 16.9[ab]	78 ± 8.9	115 ± 18.1	69 ± 10.3

[a] Difference vs. offspring of controls + offspring of non-nephropathic parents, $P < 0.01$
[bc] Difference vs. offspring of controls, [b] $P < 0.05$, [c] $P < 0.01$

Blood pressure

Sytolic, but not diastolic, blood pressures tended to be higher in the offspring of diabetic parents with nephropathy than in the offspring of diabetic parents without diabetic nephropathy (Table 2, Fig. 5). The differences in systolic blood pressures were significant when systolic BP values were compared between offspring of diabetic parents with nephropathy and controls, or controls combined with offspring of diabetic parents without diabetic nephropathy. It is of note that the difference in systolic BP tended to be higher for 24 hour BP and sleeping time BP than for casual BP. No correlation was found between any of the blood pressure indices on the one hand and night time or postexercise AER on the other hand.

Discussion

The present data clearly document increased urinary albumin excretion rates under baseline conditions as well as after physical exercise in phenotypically normal, that is, normotensive, nonsmoking, normoglycemic offspring of parents with type II diabetes and nephropathy compared to offspring of parents with type II diabetes and no nephropathy. The finding implies that subtle abnormalities of transglomerular albumin traffic are present in offspring of nephropathic diabetic patients even prior to the onset of diabetes mellitus and overt hypertension. Our results do not yield information whether the increased albumin excretion rate is related to functional or to structural changes, such as higher pressure or abnormal characteristics of the glomerular filter. As to the type of proteinuria, the higher urinary excretion of albumin in the presence of normal excretion of alpha-1-microglobulin points to a glomerular rather than a tubular problem.

When evaluated in different fashions, blood pressures were higher in offspring of diabetic parents with nephropathy, although all values remained within the normotensive range. The elevation of values concerned both casual blood pressure and even more markedly circadian blood pressure. This observation is not explained by trivial factors such as higher BMI, higher urinary sodium excretion or confounders such as use of hormonal contraception. Whether increased albumin excretion and higher blood pressures are causally related is unknown. In particular, higher postexercise albuminuria may have resulted from higher blood pressure during exercise (which was not measured). At any rate, no correlation was noted between the various parameters of blood pressure and urinary albumin excretion, but the power of the study was insufficient to be too certain about this point. Recent observations of reduced nailfold capillary density in the offspring of hypertensive parents and higher nailfold capillary pressure in prediabetic individuals [16] provide interesting conceptual links between systemic and capillary pressures, but we admit that this is purely speculative.

In a study of this nature, several potential artifacts must be considered. We made efforts to ensure that the cohort was a non-biased sample of the local population of type II diabetic patients. Type II diabetes is certainly heterogenous. We adopted the categorization according to the National Diabetes Data Group [9], and a particular effort was made to exclude MODY and mitochondrial diabetes. A problem arises because the frequency of manifest diabetic nephropathy in the parent generation must increase with duration of diabetes. As a sensible cut-off we used known duration of diabetes of ten years. We acknowledge, however, that this may have led to misclassification of some parents who may later develop nephropathy. If this is so, this would tend to obliterate any potential difference between the

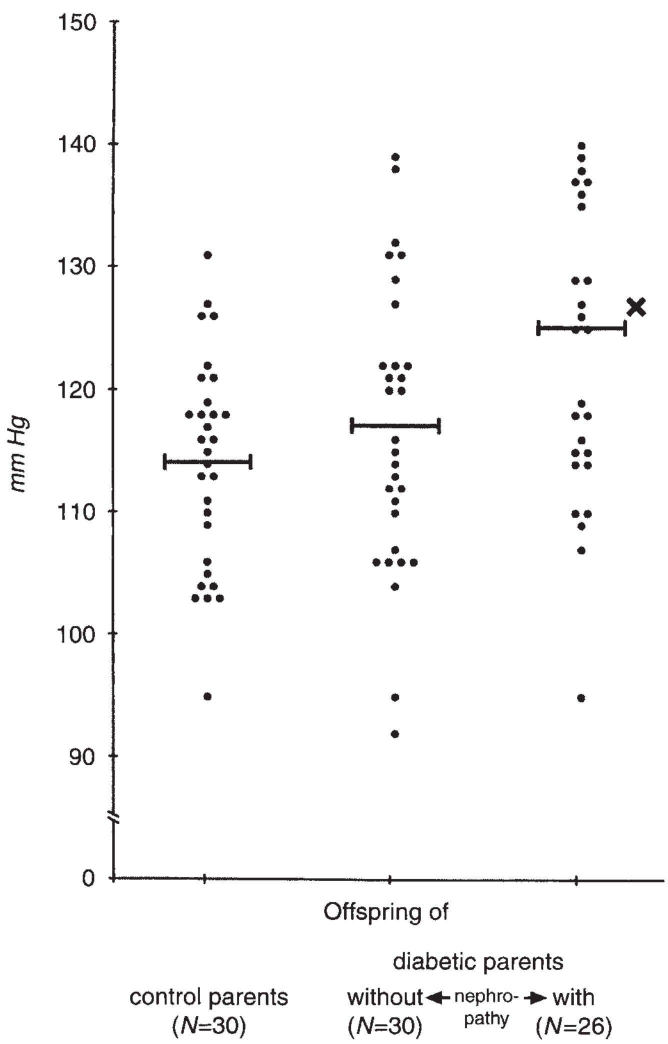


Fig. 5. *Ambulatory 24 hours systolic blood pressure in offspring of control parents and diabetic parents without and with diabetic nephropathy (DN).* Significant difference between offspring of parents with DN and controls (x $P < 0.01$).

groups of offspring and would have underestimated the genetic risk of nephropathy. We also excluded all offspring who had manifest hypertension or glucose intolerance and who were smokers or used hormonal contraception. Exclusion of such high risk offspring may have led to underestimation of the difference between groups. Indeed, hypertensive offspring of diabetic parents with nephropathy who had been excluded from the present study had higher values of AER (8.2 μg/min, range 5.3 to 18.4) compared to hypertensive offspring of non-nephropathic diabetic parents (6.1 μg/min; 4.3 to 10.1).

A family study does not allow one to distinguish between genetic factors and shared environment. Although we cannot fully exclude the latter possibility, the data would also be consistent with the operation of genetic factors. Measurements of dietary factors that are known to affect albumin excretion rate, particularly protein intake (estimated from urea excretion) and salt intake (estimated from sodium excretion) did not show differences between the groups. Furthermore, the lack of correlation between blood pressure and the albumin excretion rate would argue against, but does not fully exclude, the notion that higher AER in offspring of diabetic parents with diabetic nephropathy is merely a reflection of their higher blood pressures.

We wish to emphasize that physical exercise caused a disproportionately greater increase of urinary albumin excretion rate in offspring of parents with type II diabetes and nephropathy compared to offspring of diabetic parents without nephropathy. This is reminiscent of the dramatic increase of albuminuria by exercise in patients with type I diabetes and incipient nephropathy [17] and would be consistent with abnormal regulation of systemic and/or intrarenal hemodynamics. Whether postexercise albuminuria is predictive of later diabetic nephropathy is unknown.

The group of Campese [18] showed that individuals with salt-sensitive blood pressure tend to have higher albumin excretion rates than non-salt-sensitive individuals. We do not have data on salt sensitivity in the present cohort. This possibility requires further study, since in type I diabetes salt sensitivity of blood pressure was noted even in normotensive normoalbuminuric individuals [19].

Increased albumin excretion is associated with several facets of the "metabolic syndrome" [20, 21]. We emphasize that the offspring were all non-obese with normal glucose tolerance and normal lipid levels. Nevertheless Table 1 documents that BMI, total cholesterol and total triglyceride concentrations tended to be somewhat higher in offspring of diabetic parents, but irrespective of the presence of diabetic nephropathy in the parent generation.

Grünfeld et al [22] noted higher urinary albumin excretion in offspring of patients with essential hypertension, compared to offspring of normotensive parents, particularly after ingestion of a water and protein load. An acute increase of albumin excretion after a protein meal was also noted in the present study, but this was seen in all offspring of type II diabetic parents irrespective of nephropathy.

The present study thus confirms and extends the observation of Gruden et al [8], who noted higher albumin concentration in overnight urine in offspring of type II diabetic parents with albuminuria. The present study documents abnormal albumin excretion during stimulatory maneuvers and shows that this is associated with higher ambulatory blood pressure.

In this population we found a relationship between albuminuria and genotypes of the ACE gene, that is, 45% of subjects with D/D genotype versus 15% of subjects with I/D or I/I genotype were above the 95th percentile of morning urine albumin of controls, that is, 13 μg/min. This preliminary observation is remarkably in line with recent observations on a relationship between ACE polymorphism and microalbuminuria in essential hypertension [23] and renal prognosis in kidney disease [24–29].

It has been postulated that an abnormality of Na, Li countertransport is related to hypertension and the renal risk in diabetes [30, 31] and this line of evidence has received a more molecular basis by the recent demonstration of enhanced G protein activation of Ca^{2+} mobilization and Na/proton countertransport in patients with type I diabetes and nephropathy [32].

It has repeatedly been postulated that separate genes code for diabetes *per se* and for diabetic nephropathy [1, 4, 33]. The results of the present study would be consistent with such an hypothesis. The idea that separate genes code for, and predispose to, renal damage finds some support in recent experimental studies that use a positional cloning approach. They documented genetic determination for indicators of progressive renal dysfunction in one model of glomerulosclerosis, that is, the fawn hooded rat [34]. We admit that this interpretation of our preliminary results is speculative and that further studies are required.

The present data are in agreement with the notion that intitial changes in renal function are seen very early in the life of subjects predisposed to diabetes. Such changes may potentially determine the risk to ultimately develop diabetic nephropathy, but this remains currently unproven. Whether such early renal abnormalities have a structural basis, as postulated by one recent hypothesis [35, 36], or are of functional nature, such as higher blood pressure, abnormal intrarenal hemodynamics or abnormal glomerular permselectivity, requires further study.

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